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Gastrointestinal Biology

A comparative analysis of gastrointestinal tract barrier function and immune markers in gilt vs. sow progeny at birth and weaning

Udani A. Wijesiriwardana,[†,1] John R. Pluske,[†,‡] Jessica R. Craig,[‖] John B. Furness,[$,¶] Mitchell Ringuet,[¶] Linda J. Fothergill,[¶] Frank R. Dunshea,[†] and Jeremy J. Cottrell[†]

[†]School of Agriculture, Food and Ecosystem Sciences, The University of Melbourne, Parkville, VIC 3010, Australia
[‡]Australasian Pork Research Institute Ltd, Willaston, SA 5118, Australia
[‖]Research and Innovation, Rivalea (Australia), Pty. Ltd, Corowa, NSW 2646, Australia
[$]Florey Institute for Neuroscience and Mental Health, Parkville, VIC 3010, Australia
[¶]Department of Anatomy and Neuroscience, The University of Melbourne, Parkville, VIC, 3010, Australia

[1]Corresponding author: u.wijesiriwardana@leeds.ac.uk

Abstract

Progeny born to primiparous sows (gilt progeny; GP) have lower birth, weaning and slaughter weights than sow progeny (SP). GP also have reduced gastrointestinal tract (GIT) development, as evidenced by lower organ weights. Therefore, the aim of this experiment was to quantify changes in GIT barrier function that occur in birth and weaning, representing two major challenges to the young piglet. The effects of parity (GP vs. SP) in GIT barrier integrity function were quantified at four timepoints: birth (~0 h), 24 h after birth (24 h), 1-d preweaning (PrW), and 1-d postweaning (PoW) in commercially reared piglets. Due to inherent differences between newborn and weanling pigs, the results were analyzed in two cohorts, birth (0 vs. 24 h, $n = 31$) and weaning (PrW vs. PoW, $n = 40$). Samples of the stomach, jejunum, ileum, and colon were excised after euthanasia and barrier integrity was quantified by measuring transepithelial resistance (TER), macromolecular permeability, the abundance of inflammatory proteins (IL-8, IL-1β, and TNF-α) and tight junction proteins (claudin-2 and -3). P_{app} was characterized using a dual tracer approach comprising 4 KDa fluorescein isothiocyanate (FD4) and 150 kDa tetramethyl rhodamine isothiocyanate (T_{150})-labeled dextrans. Characteristic effects of the initiation of feeding and weaning were observed on the GIT with the initiation of feeding, such as increasing TER and reducing P_{app} at 24 h, consistent with mucosal growth ($P = 0.058$) This was accompanied by increased cytokine abundance as evidenced by elevations in TNF-α and IL-1β. However, GP had increased IL-8 abundance ($P = 0.011$ and 0.063 for jejunum and ileum respectively) at birth than 24 h overall. In the weaning cohort, jejunal and ileal permeability to FD4 was higher in GP ($P = 0.05$ and 0.022, respectively) while only higher ileal T_{150} was observed in GP ($P = 0.032$). Ileal claudin-2 abundance tended to be higher in SP overall ($P = 0.063$), but GP ileal claudin-2 expression was upregulated weaning while no change was observed in SP ($P = 0.043$). Finally, other than a higher jejunal TNF-α abundance observed in SP ($P = 0.016$), no other effect of parity was observed on inflammatory markers in the weaning cohort. The results from this study indicate that the GIT of GP have poorer adaptation to early life events, with the response to weaning, being more challenging which is likely to contribute to poorer post-weaning growth.

Lay Summary

The progeny of primiparous sows (gilt progeny; GP) have poorer lifetime growth performance in comparison to progeny from multiparous sows (sow progeny; SP). Previous research suggests that there is an underlying biological basis for reduced growth performance which is attributed to differences in gastrointestinal tract (GIT) barrier development during early life. This study aimed to clarify the timeframes of when these differences are in effect by investigating GIT development during two major events of a piglet's life: birth and weaning. To do this, GIT tissue was collected from GP and SP at four time points; birth, 24 h after birth, 1-d preweaning, and 1-d postweaning and assessed for functional development. The main findings from this study indicate there are early signs of variation in GIT development within the first 24 h of life between GP and SP, and that these differences increase through the preweaning period, with GP entering weaning with poorer GIT development and function. Possible explanations for the reduced GI development may be reduced maternal nutrition during the suckling period.

Key words: barrier integrity, gastrointestinal tract, gilt progeny, parity, sow progeny, weaning

Abbreviations: FD4, 4 kDa dextran fluorescein isothiocyanate;GIT, gastrointestinal tract; GP, gilt progeny; IL-8, interleukin-8; IL-1β, Interleukin-1β; P*app*, apparent permeability; SP, sow progeny; T_{150}, 150 kDa dextran tetramethyl rhodamine isothiocyanate; TER, transepithelial electrical resistance; TNF-α, tumor necrosis factor-alpha

Introduction

The progeny of primiparous sows (gilt progeny; GP) are characterized by their comparatively poor lifetime growth performance to those born to multiparous sows (sow progeny; SP) (Holyoake, 2006; Miller et al., 2012a; Craig et al., 2017). The reasons for their underperformance are complex and multifaceted, but recently we identified that morphologically the gastrointestinal tract (GIT) tract in GP is

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underdeveloped compared to SP when reared under commercial conditions (Craig et al., 2019). The developing piglet gut increases severalfold in weight in the first few weeks of life, with the small intestine alone increasing approximately 5-fold in weight by weaning. The perinatal period is marked by substantial remodeling due to the adaptation from placental to oral nourishment and subsequent development of digestive and barrier function. In contrast to wild-reared pigs, commercially reared piglets are abruptly weaned at 3 to 4 wk of age. While this represents stress and can be marked by disruption to the GIT, this problem is likely more acute in low birthweight piglets, for which GP are overrepresented.

Birth and weaning are two major events in a piglet's life, which can dictate their long-term health and performance, with significant growth and development occurring between these two-time points. Furthermore, these two events have major impacts on GIT structure and function required for nutrient digestion and metabolism, disease status, and growth and development (Pluske et al., 2018), all of which are imperative for long-term health of the piglet.

In a previous experiment, we compared differences in the growth and development of the GIT between GP and SP in two different cohorts, the first being 24 h after birth and the second being the peri-weaning period (Craig et al., 2019). Our findings revealed that while GP were born significantly lighter than SP, organs of the GIT such as the stomach were also significantly smaller in GP when normalized to weight, while no differences were observed in small intestinal weight and length. However, by weaning, GP had lower empty stomach weights, small intestinal weight-to-length ratios, and lower jejunal and ileal protein per gram of mucosa compared to SP. Collectively, this suggests that the disparity in GIT growth between GP and SP was largely established during the preweaning period. As a result, GP often enters weaning with a less developed GIT which may, contribute to the lighter weaning weights observed when compared to SP (Craig et al., 2019). This disparity is potentially exacerbated by the stressors imposed at weaning and results in the poorer performance of GPs throughout their lifetime.

Using the same pigs from the Craig et al. (2019) experiment, the aim of this experiment was to use GIT samples from GP to quantify the aspects of the development of epithelial barrier and localized immune function of the GIT compared to SP at birth and determine how this translates to barrier function immediately before, and after weaning.

Materials and Methods

All experimental procedures were approved by the Rivalea (Australia) Animal Care and Ethics Committee (protocol number 16P014) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

Experimental design

The following methodology is summarized by Craig et al. (2019), which details the gross morphology of tissue collected for this experiment. All experimental procedures were approved by both the Rivalea (Australia) Animal Care and Ethics Committee (16P014) and the Murdoch University Animal Ethics Committee (N2847/16) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

Tissue samples of the stomach, jejunum, ileum, and colon were taken from 71 opportunistically selected piglets (33 male and 38 female) born to 42 Large White dams (PRIMEGRO genetics, New South Wales, Australia), as previously described by Craig et al. (2019). Briefly, 33 piglets born to primiparous sows (gilts; parity 1) and 38 born to multiparous sows (parities three and four) were euthanized and GIT, with no more than two piglets per sow collected. Piglets samples were harvested at birth (0 h, before any consumption of colostrum), 24 h after birth (24 h), 24 h preweaning at approximately 29 d of age (preweaning; PrW), and at 24 h postweaning (postweaning; PoW; Table 1), henceforth called the "birth cohort" (0 and 24 h) or the "weaning cohort" (PrW and PoW).

Piglets were sedated with an intraperitoneal injection of 17 mg/kg body weight xylazine hydrochloride (Ilium Xylazil-100; Troy Laboratories Pty Ltd, NSW, Australia) and then administered an intraperitoneal anesthetic dose of 0.1 mL/kg sodium pentobarbitone (Lethabarb, Virbac, NSW, Australia), and then a jugular blood sample was collected. Piglets were then euthanized by intracardiac barbiturate overdose (0.5 mL/kg Lethabarb) and then dissected. Approximately 5 cm of fresh stomach, jejunum, ileum, and proximal colon samples (adjacent to ileocecal junction) were excised, and immediately placed into chilled 0.01M PBS (pH 7.2) for assessment of intestinal barrier function using Ussing chambers. Additionally, fresh stomach, jejunum, ileum, and colon samples were cleaned with PBS and subsequently snap-frozen in liquid nitrogen and stored at −80 °C until further analysis.

Ussing chambers

Mucosal transepithelial resistance (TER) was quantified in the stomach, jejunum, ileum, and colon, and macromolecular permeability was quantified in the jejunum and ileum as described by Cottrell et al. (2020). Briefly, after being placed into chilled PBS, the fresh tissues were opened along the mesenteric border by blunt dissection, contents removed, and placed in Krebs solution (11.1 mM glucose, 118 mM NaCl, 4.8 mM KCl, 1.0 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and pH 7.4), and continuously bubbled with carbogen (95% CO_2, 5% O_2, BOC, Australia). The mucosa was then mounted on a round slider (0.72 cm^2) and placed into a 2-part Ussing chamber (EasyMount diffusion Chambers, Physiologic Instruments, CA, USA) with 5 mL of Krebs solution added to mucosal and serosal

Table 1. Number of observations (*n*) within each dam parity (gilt progeny, GP; or sow progeny, SP), sex (male, M; or female, F) and timepoint (birth (0 h), 24 h after birth (24 h), 24 h preweaning (PrW) and 24 h postweaning (PoW)

	n			
Dam parity	**SP**		**GP**	
Sex	**M**	**F**	**M**	**F**
Timepoint				
0 h	4	5	2	4
24 h	5	4	2	5
PrW	5	5	5	5
PoW	5	5	5	5

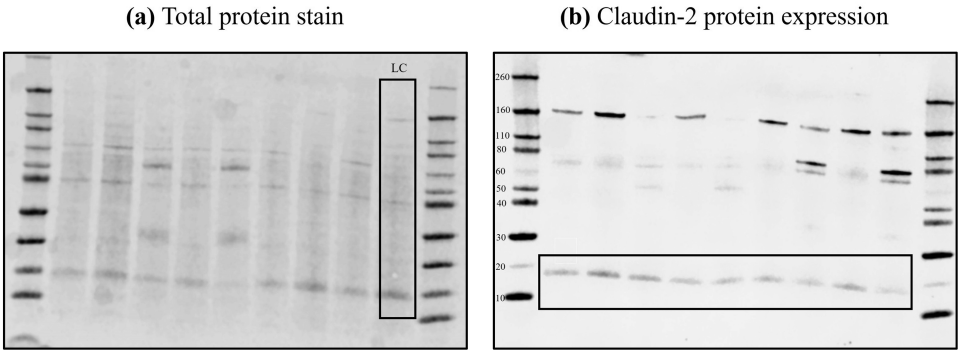

Figure 1. Western blotting images of (a) total protein stain for quantification of (b) claudin-2 in the jejunum and ileum. Protein stains were randomly allocated a lane to prevent bias during analysis. A loading control (LC) was added for inter-blot comparisons with the covariation calculated using the lane from the total protein stains.

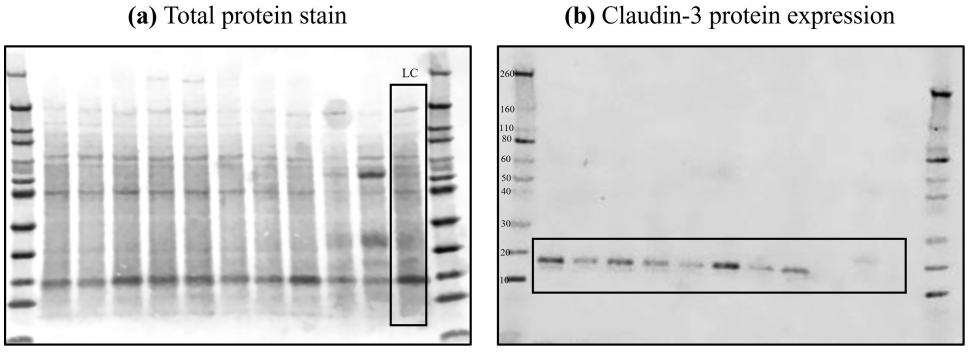

Figure 2. Western blotting images of (a) total protein stain for quantification of (b) claudin-3 in the jejunum and ileum. Protein stains were randomly allocated a lane to prevent bias during analysis. A loading control (LC) was added for inter-blot comparisons with the covariation calculated using the lane from the total protein stains.

chambers, where the glucose was replaced with mannitol in the mucosal chamber, to prevent active transport of glucose across the epithelium. A multichannel voltage-current clamp (VCC MC6, Physiologic Instruments, CA, USA) was linked to dual-voltage sensing electrodes and dual-current passing electrodes. Each electrode was submerged in 3 M KCl connected to an agar bridge (3% agarose/3 M KCl) and installed on opposite sides of the tissue. The tissue was clamped at a voltage of 0 mV and electrical currents were determined by administering 2-s stepwise pulses of 1, 2, 3, and 4 mV. Voltage and I_{sc} readings were acquired using a PowerLab amplifier and recorded using LabChart5 (AdInstruments, NSW, Australia). TER was calculated from voltage clamp steps after a 20-min equilibration period. Macromolecule permeability was assessed by measuring the flux of 50 mg/mL each of 4 kDa dextran fluorescein isothiocyanate (FD4; Sigma-Aldrich, MO, USA) and 150 kDa dextran tetramethyl rhodamine isothiocyanate (T_{150}; Sigma-Aldrich, MO, USA) from the mucosal side of the Ussing chamber to the serosal side. Two hundred µL solution from each side of the chamber was collected in triplicate at 1, 60, and 120 min and frozen at −20 °C until analysis. Concentrations were calculated using a preprepared in-house standard curve. All tissues were measured for TER and apparent permeability (P_{app}) in triplicate. The P_{app} of FD4 and T_{150} were quantified by the following equation:

$$\text{Apparent permeability } (P_{app}) = dQ/(dt \times A \times C_o)$$

Where dQ is the transport rate in mg/mL, corresponding to the linear slope of the timepoints measured (dt and timepoints). This was multiplied by the initial concentration (50 mg/mL) in the donor chamber (C_o) and the area of the slider (A, 0.72 cm^2; Tomita et al., 2004).

Western blotting

Snap-frozen whole jejunum and ileum samples were pulverized and then homogenized with radioimmunoprecipitation (RIPA) lysis buffer with protease and phosphatase inhibitors. Samples were homogenized with borosilicate 2 mm beads using a mini bead beater at 4 °C before supernatant was collected and stored at −20 °C until analysis. The BCA assay was used to standard protein concentrations to 4 µg/µL. Samples were separated by electrophoresis using 10% to 20% Tricine Protein gels (Thermo Fisher Scientific, MA, USA) under denaturing conditions. Each time a gel was prepared, the samples included were selected randomly, rather than being chosen in a predetermined manner to avoid any systematic biases related to sample placement on the gel. Following this, the gel was soaked in MilliQ water for 5 min and transferred to a 0.2 µM iBlot 2 nitrocellulose stack (Thermo Fisher Scientific, MA, USA) using the iBlot 2 system (Thermo Fisher Scientific, MA, USA) using the P3 program (20 volts, 6 min run time). Following transfer, the nitrocellulose membrane was briefly soaked in PBS and then immediately stained using LiCor REVERT total protein stain kit (LiCor, NE, USA) according to protocol and imaged using the Odessy Imaging System (LiCor, NE, USA) at 700 nm (Figure

1a, Figure 2a). Membranes were washed with MilliQ water and then blocked in 5% nonfat dry milk (NFDM) in TBST (1 × Tris Buffered Saline, 1 % Tween-20) for 2 h. Membranes were then incubated with primary antibodies claudin-2 and claudin-3 (Product Number 32-5,600 and 34-1,700, respectively, Thermo Fisher Scientific, MA, USA) with 5% NFDM in TBST overnight with dilutions 1:250 and 1:750, respectively, (Figure 1b, Figure 2b). Following this, the membrane was washed three times with TBST for 5 min and then incubated with IRDye 800CW secondary antibodies (Product Number 925-32,213, LiCor, NE, USA) in 5% NFDM in TBST for 1 h. After incubation steps, the membrane was washed three times with TBST for 5 min. Following final washing, the membrane was imaged using infrared fluorescence of the Odessy Imaging System at 800 nm. Both images of the total protein stain and antibody stain were analyzed using Licor Image Studio (LiCor, NE, USA). A loading control of pooled jejunum homogenate was used on each blot in order to normalize all blots to each other. Samples that were measured at 0 AU were labeled as not detected and removed from the dataset.

Inflammatory marker analysis

Approximately 100 µg of snap-frozen whole jejunum and ileum tissue was weighed and homogenized in lysis buffer as previously described by Helm et al. (2019) with borosilicate 2 mm beads (Sigma-Aldrich, MO, USA) using a mini bead beater (BioSpec, OK, USA). Samples were then centrifuged at 15,000 × *g* for 10 min at 4 °C and the supernatant was collected. The supernatant from each sample was analyzed by ELISA for interleukin-8 (IL-8), tumor necrosis factor- α (TNF-α), and interleukin-β (R&D Systems, MN, USA). Samples were assayed as per the manufacturer's guidelines with the exception that tissue supernatant was used rather than plasma as outlined by Pearce et al. (2013). Assays were performed in duplicate with 100 µg/well of protein loaded into each well. Protein concentrations were determined using a BCA protein assay. The intra-assay and inter-assay CV for IL-8 were 8-19% and 9%, respectively, for TNF-α, 3% to 7% and 15%, respectively, and for IL-1β, 4% to 6% and 5%, respectively.

Statistical analyses

Data were analyzed using a linear mixed model in RStudio (2023.03.0 Build 386; Boston, USA). Data from both the birth and weaning cohorts were analyzed separately. Parity (gilt vs. sow) and time within cohorts (birth; 0 h vs. 24 h and weaning; PrW vs. PoW) were analyzed as the main and interactive effects for intestinal integrity and inflammation markers. Where main or interactive effects were found to be statistically significant, post hoc analysis using Tukey's HSD test. Data were checked for normality using the Shapiro–Wilk test. If the test returned $P < 0.05$, data were considered not normal and subsequently transformed by the natural logarithm (ln) and analyzed as above. Data are presented as the pooled means for the main effects of parity, time, and the standard error of the difference (SED). Means were considered significantly different at $P < 0.05$ and $P < 0.10$ was considered a trend.

Results

Birth cohort

Mucosal TER and permeability

Stomach TER was higher in SP than in GP overall (101 vs. 72.9 Ω.cm^2, $P = 0.015$; Table 2). The TER declined over the first 24 h after birth in the stomach (98.5 vs. 76.1 Ω.cm^2, $P = 0.048$) and colon (130 vs. 100 Ω.cm^2, $P = 0.004$), but increased in the jejunum (24.4 vs. 34.5 Ω.cm^2, $P = 0.004$) and ileum (26.5 vs. 38.4 Ω.cm^2, $P = 0.002$). An increase in jejunum FD4 (473 vs. 1,492 × 10^{-4} cm·s^{-1}, respectively, $P = 0.003$) and T_{150} (73.2 vs. 371 × 10^{-4} cm·s^{-1} respectively, $P = 0.001$) permeability was observed between 0 and 24 h. No main or interactive effects were observed in the ileum for FD4 and T_{150} permeability (Table 2).

Tight junction protein expression

No main or interactive effects were observed on claudin-2 expression in the jejunum (Figure 3a). However, claudin-3 concentrations in the jejunum tended to be higher in SP compared to GP (4.36 vs. 2.51 AU, $P = 0.058$; Figure 3b). No significant main or interactive effects were observed on claudin-2 and -3 expression in the ileum (Figure 3c, d).

Cytokine abundance

The IL-8 concentrations in the jejunum were higher in SP compared to GP (5.67 vs. 4.14 pg/µg, $P = 0.011$) and declined from 0 to 24 h (5.68 vs. 4.12 pg/µg, $P = 0.002$; Table 3). The main effect of time was present for IL-1β such that its concentration increased from 0 to 24 h (0.78 vs. 1.20 pg/µg, $P = 0.017$; Table 3). Jejunal TNF-α concentrations tended to be higher in SP compared to GP (2.72 vs. 0.12 pg/µg, $P = 0.087$) and increased from 0 to 24 h (1.20 vs. 0.78 pg/µg, $P = 0.017$; Table 3). No other significant main or interactive effects were observed in jejunal cytokine abundance (Table 3).

Concentrations of IL-8 in the ileum tended to be lower in SP compared to GP (2.74 vs. 4.09 pg/µg, $P = 0.063$) and decreased from 0 h compared to 24 h (4.26 vs. 2.54 pg/µg, $P = 0.003$; Table 3). The TNF-α concentrations increased from 0 h to 24 h (0.66 vs. 1.12 pg/µg, $P = 0.008$). No other significant main or interactive effects were observed in ileal cytokine abundance (Table 3).

Weaning cohort

Mucosal TER and permeability

The TER of the stomach (102 vs. 82 Ω.cm^2, $P = 0.014$) and jejunum (51.0 vs. 36.4 Ω.cm^2, $P = 0.003$) declined from PrW to PoW. The SP tended to have higher TER in the ileum compared to GP (76.9 vs. 67.9 Ω.cm^2, $P = 0.099$; Table 4). Furthermore, TER of the ileum tended to decline from PrW to PoW (77.8 vs. 67.0 Ω.cm^2, $P = 0.066$; Table 4). An interaction between parity and time was observed such that colon TER was higher at PrW than PoW in GP, while no effect was observed in SP ($P = 0.002$; Table 4).

SP had lower jejunal permeability to FD4 compared to GP (321 vs. 700 × 1^{-4} cm·s^{-1}, $P = 0.050$; Table 4). Furthermore, SP had lower ileum permeability towards FD4 (146 vs. 293 × 10^{-4} cm·s^{-1}, $P = 0.03$) and T_{150} (41.2 vs. 97.1 × 10^{-4} cm·s^{-1}, $P = 0.01$) compared to GP. No other significant main or interactive effects were observed (Table 4).

Tight junction protein expression

No main or interactive effects were observed on jejunal claudin-2 expression (Figure 4a). No jejunal claudin-2 was detected in GP in the PrW group. There was no significant effect of parity on jejunal claudin-3 expression (Figure 4b). However, jejunal claudin-3 significantly declined after

Table 2. The effect of parity (P; sow progeny; SP vs. gilt progeny; GP), time (T; birth; 0 h vs. 24 h after birth; 24 h) and their interaction (P × T) on transepithelial resistance (TER, Ω.cm²) and FD4 and T_{150} apparent permeability (P_{app}, × 10^{-4} cm · s^{-1}) on dissected GIT organs. Standard error of differences presented as SED

	Factor							
	SP		GP			*P*-value		
Parameter	0 h	24 h	0 h	24 h	SED	P	T	P*T
TER, Ω.cm²								
Stomach	120	83.2	76.8	69.1	16.9	0.015	0.048	0.23
Jejunum	25.0	33.9	23.7	35.2	4.5	0.78	0.004	0.68
Ileum	25.5	41.6	27.6	35.3	5.3	0.73	0.002	0.27
Colon	127	99.4	133	102	13.3	0.94	0.004	0.84
P_{app}, ×10^{-4} cm·s^{-1}								
Jejunum								
FD4[1] ln	6.02	7.20	6.28	7.07	0.48	0.59	0.003	0.33
	(412)	(1808)	(533)	(1176)				
T150 ln	3.87	6.10	4.59	5.69	0.64	0.23	0.001	0.23
	(47.9)	(446)	(98.5)	(296)				
Ileum								
FD4 ln[1]	5.80	5.19	5.17	5.70	0.75	0.87	0.91	0.14
	(330)	(179)	(176)	(299)				
T150 ln[1]	4.50	4.22	4.28	3.93	0.62	0.36	0.32	0.91
	(90.0)	(68.0)	(72.2)	(50.9)				

[1]Due to sample heterogeneity the values were transformed using the natural log (ln). Values were then back-transformed and presented in parentheses for comparison purposes.

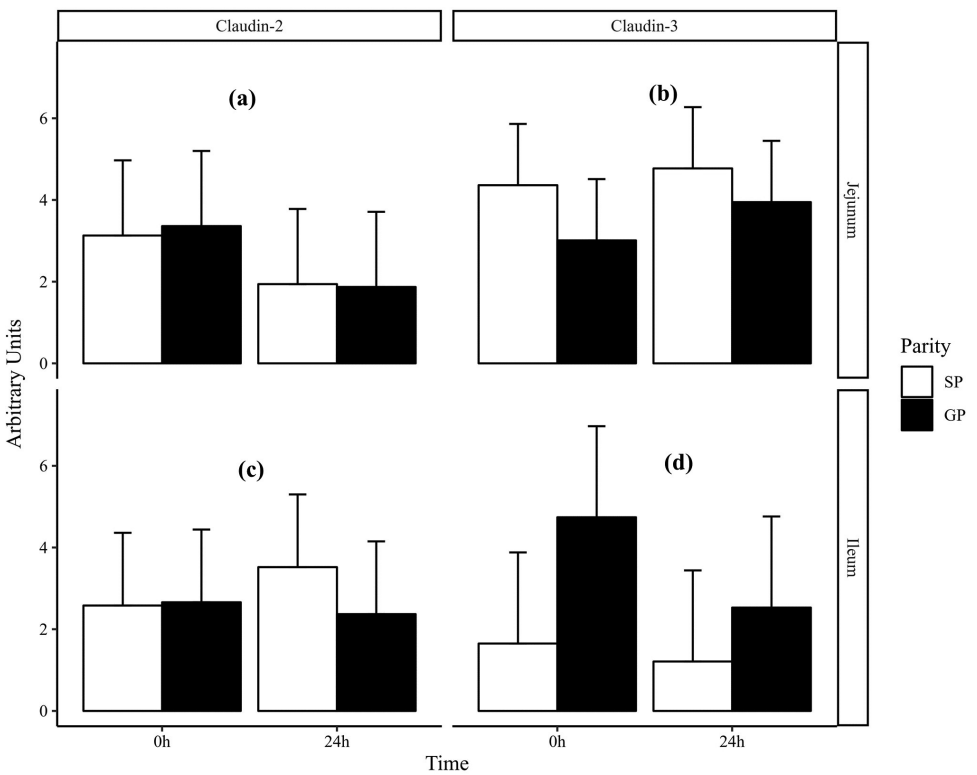

Figure 3. The effect of parity (sow progeny; SP vs. gilt progeny; GP) and time (birth; 0 h vs. 24 h after birth; 24 h) on jejunum (a) claudin-2 and (b) claudin-3 expression and ileum (c) claudin-2 and (d) claudin-3 expression. Data in figures are presented as natural log-transformed means and standard error of the transformed mean for the Parity (P) × Time (T) interaction. The *P*—values for the main and interactive effects of P and T were (a) 0.81, 0.15 and 0.87 (b) 0.058, 0.79 and 0.75 (c) 0.60, 0.79 and 0.51 and (d) 0.18, 0.36 and 0.51.

Table 3. The effect of parity (P; sow progeny; SP vs. gilt progeny; GP), time (T; birth; 0 h vs. 24 h after birth; 24 h) and their interaction (P*T) on concentrations (pg/μg) of interleukin-8 (IL-8), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the jejunum and ileum. Standard error of differences presented as SED

	Factor							
	SP		GP			*P*-value		
Parameter	0 h	24 h	0 h	24 h	SED	P	T	P*T
Jejunum, pg/μg								
IL-8	4.78	3.49	6.60	4.74	0.65	0.011	0.002	0.54
IL-1β ln[1]	−1.02	0.30	−1.53	0.06	0.79	0.68	0.017	0.82
	(0.36)	(1.35)	(0.22)	(1.06)				
TNF-α	0.12	3.41	0.12	2.02	0.81	0.087	<0.001	0.28
Ileum, pg/μg								
IL-8	3.17	2.25	5.35	2.83	0.70	0.063	0.003	0.12
IL-1β ln[1]	−0.57	−0.28	−0.52	−0.31	0.58	0.84	0.51	0.91
	(0.57)	(0.76)	(0.60)	(0.73)				
TNF-α	0.03	3.05	0.04	1.12	1.33	0.16	0.008	0.34

[1]Due to sample heterogeneity the values were transformed using the natural log (ln). Values were then back-transformed and presented in parentheses for comparison purposes.
[a-b]Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 4. The effect of parity (P; sow progeny; SP vs. gilt progeny; GP), time (T; 1 d preweaning; PrW vs. 1 d postweaning; PoW) and their interaction (P*T) on transepithelial resistance (TER, Ω.cm^2) and FD4 and T_{150} apparent permeability (P_{app}, × 10^{-4} cm · s^{-1}) on dissected GIT organs. Standard error of differences presented as SED

	Factor							
	SP		GP			*P*-value		
Parameter	PrW	PoW	PrW	PoW	SED	P	T	P*T
TER, Ω.cm^2								
Stomach	100	77.0	104	87.0	11.0	0.36	0.014	0.69
Jejunum	54.2	39.6	47.8	33.1	6.6	0.17	0.003	0.99
Ileum	86.9	66.9	68.8	67.1	7.9	0.099	0.066	0.11
Colon	58.5[b]	69.2[a,b]	82.7[a]	57.1[b]	7.5	0.14	0.17	0.002
P_{app}, ×10^{-4} cm·s^{-1}								
Jejunum								
FD4[1] ln	6.50	6.60	5.86	5.67	0.54	0.050	0.90	0.71
	(665)	(735)	(351)	(290)				
T_{150} ln	4.28	4.28	4.71	4.21	0.67	0.54	0.47	0.47
	(72.2)	(72.2)	(111)	(67.4)				
Ileum								
FD4 ln[1]	4.73	5.18	5.57	5.78	0.62	0.03	0.30	0.71
	(113)	(178)	(262)	(323)				
T_{150} ln[1]	3.88	3.52	4.32	4.78	0.61	0.01	0.87	0.19
	(48.4)	(33.9)	(75.2)	(119)				

[1]Due to sample heterogeneity the values were transformed using the natural log (ln). Values were then back-transformed and presented in parentheses for comparison purposes.
[a-b]Values within a row with different superscripts differ significantly at $P < 0.05$.

weaning (3.45 vs. 1.35 AU, $P = 0.03$; Figure 4b). No interactive effects were observed in jejunal claudin-3 expression (Figure 4b). SP tended to have higher ileal claudin-2 expression compared to GP (2.92 vs. 2.26 AU, $P = 0.069$; Figure 4c), while no main effect of time was observed (Figure 4c). No significant main or interactive effects were observed for ileum claudin-3 expression (Figure 4d).

Cytokine abundance

Jejunal TNF-α concentrations were higher in SP than GP (0.34 vs. 0.21, $P = 0.016$) while no other main or interactive effects of parity or time were observed in the jejunum (Table 5). Furthermore, no significant main or interactive effects were observed in IL-8, IL-1β, and TNF-α in the ileum (Table 5).

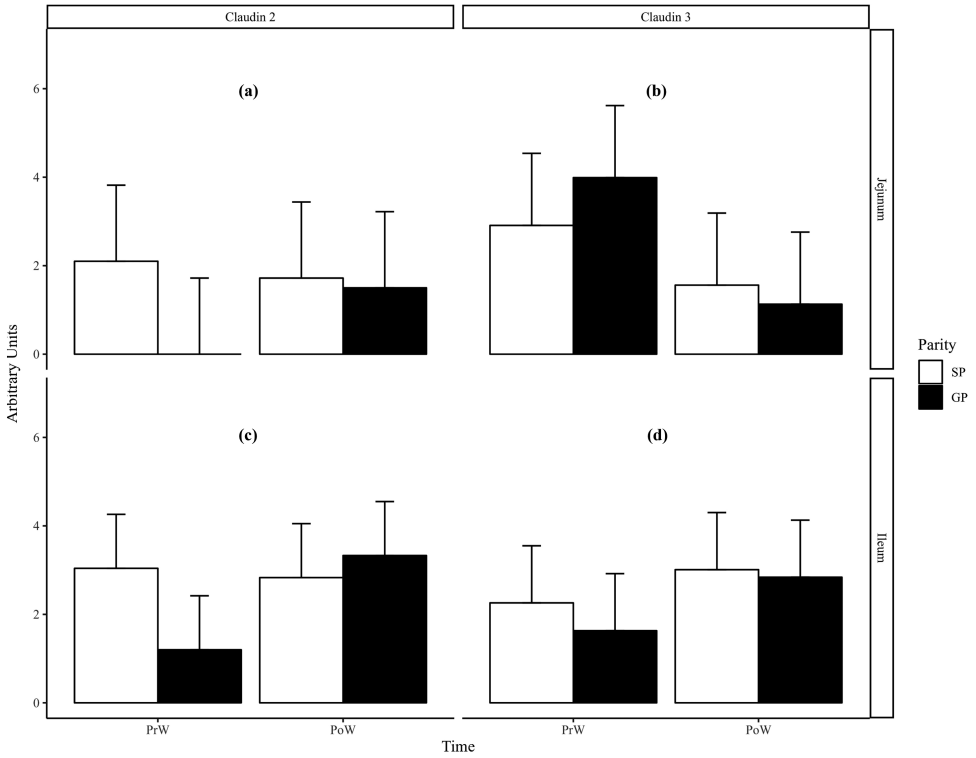

Figure 4. The effect of parity (sow progeny; SP vs. gilt progeny); GP and time (1 d preweaning; PrW vs. 1 d postweaning; PoW) on jejunum (a) claudin-2 and (b) claudin-3 expression and ileum (c) claudin-2 and (d) claudin-3 expression. Data in figures are presented as natural log-transformed means and standard error of the transformed mean for the Parity (P) × Time (T) interaction. The *P*—values for the main and interactive effects of P and T were (a) 0.18, 0.39 and 0.18 (b) 0.97, 0.03 and 0.41 (c) 0.069, 0.11 and 0.043 (d) 0.40, 0.23 and 0.77.

Discussion

Gilt progeny are characterized by their low birth weights and weaning weights in comparison to SP. These differences may in part, be linked to complex underlying biological mechanisms. The primary objective of this study was to quantify GIT development with particular emphasis on potential differences in maturation of GIT barrier function and localized cytokine profiles between GP and SP under commercial farming conditions. The main findings from this study show differences in intestinal barrier function between GP and SP are minimal at birth but manifest at weaning, suggesting reduced maturation of the GIT lining during the nursing period.

Assessment of intestinal barrier function in neonatal GP and SP using a dual-marker approach

In this study, the neonatal period was marked by an increase in TER in the jejunum and ileum between 0 and 24 h and an increase in jejunal permeability towards FD4 and T_{150} (4 kDa and 150 kDa-sized macromolecules) for both GP and SP. This elevated jejunal permeability at 24 h suggests that "gut closure", traditionally understood as a reduction in intestinal permeability in response to colostrum consumption, extends beyond the 24 h time. Indeed, this aligns with previous studies where colostrum consumption has been reported to stimulate a reduction in permeability approximately 24 to 36 h postnatally (Speer et al., 1959; Lecce and Morgan, 1962; Weström et al., 1984a). Speer et al. (1957) also observed intestinal closure to IgG taking place as early as 24 h of age in suckling piglets.

While our methodology used a dual-marker approach to establish a correlation between elevated permeability at 24 h in the jejunum and colostrum consumption, it is essential to note that the epithelium may have distinct transport mechanisms for specific molecules (Sangild, 2003). Nonetheless, previous studies (Weström et al., 1984b; Jensen et al., 2001; Varadarajan et al., 2019) show that intestinal permeability to IgG and these macromolecules exhibit degrees of similarity in the timing of their movement across the epithelium in this neonatal window.

Overall, the changes in intestinal barrier function in the birth cohort of our study are consistent with previously noted correlations between early intestinal development and colostrum consumption intake (Simmen et al., 1990; Burrin et al., 1992). Furthermore, our prior study (Craig et al., 2019) indicated increases in small intestinal weight and length in the preweaning period. Collectively, these observations demonstrate the ability of colostrum consumption to stimulate early intestinal barrier development.

Transepithelial resistance in the stomach and colon of the neonatal pig

Previously, we observed that absolute stomach weights were higher in SP than GP overall in the birth cohort (Craig et al., 2019). In the present study, TER was higher in SP than GP overall. As TER was measured on stripped mucosa, this result may indicate that SP had a higher amount of ion channels or tight junction integrity, or the mucosal thickness of the epithelial lining was higher in SP than GP which is reflected in their higher absolute stomach weights. A reduction in stomach TER from 0 to 24 h was observed in both GP and SP, and

Table 5. The effect of parity (P; sow progeny; SP vs. gilt progeny; GP), time (T; 1 d preweaning; PrW vs. 1 d postweaning; PoW) and their interaction (P*T) on concentrations (pg/μg) of interleukin-8 (IL-8), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the jejunum and ileum

	Factor							
	SP		GP			*P*-value		
Parameter	PrW	PoW	PrW	PoW	SED	P	T	P*T
Jejunum, pg/μg								
IL-8	8.53	9.54	8.97	10.8	1.49	0.37	0.18	0.69
IL-1β ln[1]	−2.49	−2.50	−2.05	−2.62	1.17	0.85	0.66	0.64
	(0.08)	(0.13)	(0.13)	(0.07)				
TNF-α	0.31	0.38	0.22	0.20	0.08	0.016	0.73	0.42
Ileum, pg/μg								
IL-8	10.5	8.52	8.82	11.20	2.13	0.67	0.85	0.16
IL-1β ln[1]	−0.72	−1.42	−0.83	−0.73	0.60	0.27	0.31	0.19
	(0.49)	(0.24)	(0.44)	(0.48)				
TNF-α	0.17	0.33	0.17	0.15	0.12	0.21	0.45	0.31

[1]Due to sample heterogeneity the values were transformed using the natural log (ln). Values were then back-transformed and presented in parentheses for comparison purposes.
Standard error of differences presented as SED.

most likely reflects increases in ionic permeability associated with digestion, such as the 0secretion of bicarbonate-rich mucous. Furthermore, a reduction in colonic TER was also observed from 0 to 24 h in both parity groups. At 0 h the meconium was present in all piglets and as it was adherent to the colonic mucosa it was left in place as it could not be removed without damaging the colonic mucosa. In the 24 h sample, the meconium had typically shed or was loosely bound and therefore was removed for consistency. Therefore, the effects of the initiation of feeding on the colonic mucosa are confounded by the process of meconium shedding, but it is possible the reduction in TER reflects the shedding of the meconium and not an underlying change in the colonic mucosa.

Cytokine abundance in the small intestine of the birth cohort

There was an overall parity effect across the birth cohort whereby elevated levels of IL-8 were observed in the jejunum and ileum as well as a trend for increased jejunum TNF-α in GP. Collectively, this indicates an elevated inflammatory response in the small intestine of GP from birth. Interleukin-8 is a pro-inflammatory cytokine due to its neutrophil chemoattractant property, responsible for recruiting neutrophils to sites of injury or infection. However, Maheshwari et al. (2002) reported that IL-8 can exert other effects in the human fetal GIT such as stimulating maturation without the influx of neutrophils. However, as differences between GP and SP were minimal in the birth cohort, the specific role of IL-8 in this instance may not exclusively involve stimulating the maturation of the small intestine of GP. Consequently, the reasons behind the elevated levels of IL-8 in the intestines of GP are unclear and warrant further investigation.

Higher concentrations of jejunal IL-1β and TNF-α and ileal TNF-α were observed at 24 h in both GP and SP and may be attributed to the transfer of these cytokines via colostrum (Nguyen et al., 2007). Unlike the current study, Nguyen et al. (2007) reported undetectable concentrations of serum TNF-α after consuming colostrum. The difference between the findings could be attributed to the short half-life of this cytokine (Liu et al., 2021) and its being present at a higher concentration in serum compared to direct absorption by the intestine. To the best of our knowledge, the concentrations of IL-1β in sow colostrum have yet to be elucidated, but results from this study indicate a similar transfer of IL-1β as to TNF-α. In this respect, further work in characterizing lactogenic cytokines in sow and gilt colostrum is warranted.

The effect of weaning on intestinal barrier function in SP vs. GP

When mucosal barrier function was assessed at weaning, a reduction in the TER of the stomach and small intestine and jejunal claudin-3 expression in both GP and SP indicated a compromised barrier function in the proximal section of the intestine in response to weaning. Interestingly, GP exhibited a lower ileal TER overall at weaning accompanied by a significant reduction in colonic TER from PrW to PoW. These observations suggest that GP may experience an exacerbated impact of weaning on the distal portion of the intestine when compared to SP.

Our study observed an increase claudin-2 expression in GP PoW, indicating a potential association with weaning claudin-2 has a primary role in facilitating ion secretion and mediating water transport (Amasheh et al., 2002), critical for the proper function of the intestine and therefore, this finding in conjunction with the reduced ileal from PrW to PoW seems contradictory. However, it has been reported that an upregulation of claudin-2 expression, specifically in response to stress or low-grade inflammation, can cause reduced epithelial integrity (Ishimoto et al., 2017). Liu et al. (2013) reported that corticotropin-releasing factor (CRF) can cause increased expression of claudin-2 in human cell lines, which reduces TER in epithelial monolayers. Low-grade inflammation and increased concentrations of CRF reflect the stressors encountered at weaning (Moeser et al., 2007) and, therefore, may explain the increased claudin-2 expression in GP observed in this study in response to weaning. While higher claudin-2 expression was seen in SP overall, this could be attributed to

overall greater intestinal function development in SP. Furthermore, the higher claudin-2 expression of SP did not seem to negatively affect intestinal barrier function of SP to the same extent as GP at weaning (i.e., poorer intestinal function and growth after weaning). However, since CRF was not measured in this study and claudin-2 expression in response to CRF has mainly been analyzed in cultured epithelial monolayers, this is speculative, and more research is required in this area.

In the current study, GP also exhibited highly permeable ileal tissue to both FD4 and T_{150} in addition to lower TER at weaning. Furthermore, colonic TER was also reduced from PrW to PoW specifically in GP. Taken together, this suggests a higher susceptibility towards leakiness in the colon (Moeser et al., 2007) and the propensity for an increase in luminal antigens entering circulation, promoting a higher incidence of postweaning infections (Miller et al., 2012b) and slower growth observed in GP (Wijesiriwardana et al., 2020).

Findings from this study show that GPs have a lower intestinal barrier function than SP at weaning. This aligns with their characteristic slower growth in the preweaning phase (Craig et al., 2017) and implies that GP may ingest less colostrum or milk during this period. Indeed, it has been reported that piglets fostered onto primiparous sows consumed less and were consistently lighter than those fostered on multiparous sows (Ferrari et al., 2014). Colostrum and milk are rich sources of growth factors such as epidermal growth factor, insulin-like growth factors I and II, insulin, and transforming growth factor-β which are critical for stimulating the growth and functional maturation of intestinal tissues (Xu et al., 2000). Lower colostrum and/or milk consumption in GP may explain both their slower preweaning growth and the biological mechanisms underlying this lag (i.e., their lower TER in the distal portions of the intestine). Collectively, this can have long-term impacts on their overall growth, development, and health status throughout life. For example, Lessard et al. (2018) reported that slow-growing piglets have reduced immunocompetence, which is in line with results from this study where lower jejunal concentrations of TNF-α were observed in GP compared to SP at weaning which may impeded their ability to cope with the stressors of weaning. Indeed, higher mortality and medication rates have been observed in GP following weaning (Craig et al., 2017). Findings from this study suggest that differences in GIT development between GP and SP occur during the preweaning period when reared under commercial conditions.

The findings from the present study found that while GP and SP showed minimal differences in GIT barrier function and localized cytokine profiles suggesting that GIT development is not compromised during fetal development. However, we observed reductions in the development of these characteristics during the lactation period in GP, when reared under commercial conditions. Taken together, we suggest that differences in intestinal development occur throughout lactation and barrier function of the intestine in GP is more influenced by weaning than SP. This may be a contributing factor to the increased prevalence of postweaning diarrhea found in GP. Therefore, we suggest that management strategies aimed at supporting higher milk yield and/or increasing sow longevity in the breeding herd and thereby, minimizing turnover and the proportion of piglets born to primiparous sows may mitigate these effects.

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Conflict of Interest Statement

We declare no conflicts of interest.

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